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Impact of Epidermal Thickness on Purpura From the Pulsed Dye Laser

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Background and Objective: **To clarify whether epidermal thickness is of importance to the purpuric reaction from treatment with the pulsed dye laser (PDL).**
Study Design/Materials and Methods: **Fifteen fairly pigmented volunteers were laser treated in two test regions of varying epidermal thicknesses: normal buttock skin and ultraviolet B (UVB)-exposed buttock skin. Laser treatments were performed with the flashlamp-pumped PDL (585 nm). Fluences ranged from 3–6.5 J/cm^2, spot size was 7 mm, and each volunteer received at least six fluences in each treatment region. Assessment of the response was based on clinical evaluation (threshold dose to purpura 10 minutes and 1 day after treatment) and skin reflectance-evaluated redness (1 and 6 days, 2 and 6 weeks after treatment).**
Results: **The total epidermal thickness differed between the unexposed buttock skin (median, 72.7 μm) and the UVB-exposed buttock skin (87.2 μm) (*P* < 0.01). There was no correlation between the epidermal thickness and the threshold dose to induce purpura 10 minutes and 1 day after laser exposure. Skin reflectance revealed no correlation between the epidermal thickness and the skin reflectance evaluated redness on 1, 6 days, and 2 weeks postoperatively. A dose-response relation was seen within the two test regions; 6 weeks after laser exposure, there was no remaining laser-induced skin redness.**
Conclusion: **The epidermal thickness is unimportant to the purpuric reaction after PDL treatment. Lasers Surg. Med. 22:159–164, 1998.**

Key words: epidermis; experimental study; laser surgery; UV radiation

INTRODUCTION

The pulsed dye laser (PDL) is today considered the treatment of choice for portwine stains (PWS), especially in the pediatric population [1,2]. PWS are congenital vascular malformations, which most commonly involve the face and neck and are characterized by ectatic capillary and venule-size vessels in the papillary and upper reticular dermis [3]. The PDL is based on the concept of selective photothermolysis (585 nm, 450 μsec) that stands for a precise targeting of the dilated dermal vessels [4].

It is well known that the efficacy of the PDL displays an inter- and intraindividual variation and that the treatment response is unpredictable for the individual patient. Among several factors, the diameter and the depth of the targeted vessels

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have been associated with variation in treatment outcome [5–7].

Variations in epidermal thickness also may influence the penetration of the laser light into the dermal layers, since a thick epidermis may attenuate the laser light before it targets the underlying, dermal vessels. We have previously presented a murine study outlining that a thick epidermis reduces the laser-induced side effects from treatment with the copper vapor laser (CVL, 578 nm) [8]. However, it is unknown whether the epidermal thickness influences the clinical response from treatment with the PDL.

From a clinical point of view, it is relevant to consider the importance of variations in epidermal thickness to the efficacy of the PDL, since vascular lesions are located in anatomical regions of varying epidermal thickness and since exposure to sunlight increases the epidermal thickness [9]. The interaction between UV irradiation and laser light is important, since the majority of vascular lesions are located in UV-exposed skin areas [3].

The aim of the present study was, therefore, to clarify whether epidermal thickness is of importance to the purpuric reaction that is induced by the PDL.

MATERIALS AND METHODS

Subjects and Protocols

Fifteen healthy volunteers (range 19–31 years) were enrolled in the study after giving informed consent. The skin type classification system described by Fitzpatrick [10] was used in which white skin types are divided into four classes. According to that classification 10 volunteers had skin type I and 5 had skin type II.

All volunteers were laser treated in two test regions of varying epidermal thicknesses. Test regions included: (1) unexposed normal buttock skin, and (2) UVB-exposed buttock skin. Four days passed from the last UV irradiation to the laser treatment in order to eliminate UV-induced acute inflammation, but to maintain the induced epidermal thickening. Superficial biopsies were taken immediately after laser treatment from adjacent, unexposed skin in order to measure the epidermal thicknesses in the test regions. Assessment of the response to therapy was based on clinical evaluations, using blinded photographic documentation, and on skin reflectance measurements.

UV Exposure and Radiation Source

All volunteers were exposed to UV eight times during 3 weeks. UV exposure was given to one test area (6 × 10 cm) on the buttock of the volunteers. During the first week the volunteers received two UV exposures; during the second and third weeks they received three UV exposures. UV radiation was obtained from Phillips TL 01 tubes, which emits UVB in a narrow peak ~312 nm. The intensity of the source was measured as previously described [11]. The three first UV exposures were equivalent to 0.75× the original minimal erythema dose (MED), the following two equivalent to 1× the original MED, and the last three to 1.25× the original MED. The physical doses to one MED varied among the volunteers; the median dose to one MED was 0.41 J/cm^2. Every UV exposure was below the present erythema level for the volunteers. However, the cumulative effect of the daily successive UV exposures occasionally induced erythema. Skin pigmentation was followed by skin reflectance in every volunteer during the 3-week UV course.

Laser Techniques

A commercially available flashlamp-pumped pulsed dye laser (Candela SPTL-1b, Candela Corp., Wayland, MA), with a wavelength of 585 nm ± 5 nm and a pulse duration of 450 μs, was used. The spot diameter was 7 mm, which according to the manufacturer's manual was ±0.5 mm. The laser spots were applied as single spots without overlap. The energy fluences ranged from 3–6.5 J/cm^2 with 0.5 J/cm^2 increments. An external energy meter (Ophir PE50-DIF) calibrated to ±3% was used to monitor fluences. In the laser surgery room, the temperature was between 22°C and 24°C. Laser treatments were performed in the two test regions; six fluences were used and each fluence was delivered as four single pulses. Occasionally, additional fluences were delivered if it had not been possible to establish the threshold dose to cause purpura with the predicted doses. No local, anaesthesia was used during the laser treatments.

Thicknesses of Stratum Corneum and Cellular Part of Epidermis

Light microscopy was used to measure the thickness of stratum corneum and of the cellular part of epidermis. Superficial biopsies were taken from the three test regions ($n = 29$, area ~4 mm^2). Specimens were snap frozen, fixed in isopentane

(2-methylbutan), and stored at −80°C. Sections were cut at 8 μm and stained with haematoxylin and eosin. The section of the highest technical quality out of 5–10 sections from one biopsy was evaluated, and at least four evaluations were performed per section with the purpose of obtaining representative mean values. Measuring the thickness of the cellular part of epidermis was based on an area-measurement by means of a calibrated square grid as previously described, stratum corneum was measured by means of a calibrated ocular micrometer that was placed perpendicular to the basis of the epithelium [8]. The thickness of the entire epidermis was calculated by adding corresponding thicknesses of stratum corneum and the cellular part of epidermis.

Evaluation of Skin Reactions

The assessment of the response to therapy was based on clinical establishment of the threshold dose to induce purpura, using blinded photographic evaluation and skin reflectance measurements.

The purpura threshold dose was established 10 minutes after laser exposure, and 1 day postoperatively as the minimum fluence, which in three out of the four single spots could induce the presence of nonblanchable purpura filling the whole spotsize. The assessment of the purpura threshold dose was made independently and blinded by two doctors based on photographic documentation. All photographs were taken by a single photographer using the same camera (Nikon F70), film type (Kodak, Ektachrome 100), lighting conditions (TTL auto flash shooting; Twin Flash Nikon Macro Speedlight SB-21/Flash Terminal Nikon AS14), camera settings (distance 0.35 m, aperture 11, body focusing technique), and film processing technique (E-6).

Skin reflectance was used to quantify skin redness and pigmentation (UV-Optimize, Matic, Herlev, Denmark). The equipment uses 555 and 660 nm wavebands of light where the discrimination between light absorption in melanin and hemoglobin is maximal. Skin pigmentation and redness are quantified independently and continuously on relative scales from 0–100% [12]. Zero percent pigmentation is found in white skin with no pigment at all, and 100% corresponds to the pigmentation in theoretically absolutely black skin with no light reflection. Zero percent skin redness corresponds to skin where blood has been temporarily drained from the area; 100% redness corresponds to highly vascular tissue with a dark bluish red PWS. The redness% in the laser treated skin was corrected for fluctuations in the vascular bed by subtracting the redness% of the surrounding normal skin. The measurements were performed before the laser treatment, as well as 1 and 6 days, 2 and 6 weeks after the treatment. No measurements were performed 10 minutes after laser treatment because of laser-induced oedema.

Laser Doppler Flowmetry

Laser doppler flowmetry has been described in detail and is recognized as a noninvasive technique to observe microcirculatory flow [13]. Cutaneous perfusion was assessed immediately before laser exposure in the two test regions in order to elucidate that the UV-induced inflammation had been eliminated at the time of laser exposure (Laser Blood Flow Monitor MBF3, Moor Instruments, UK).

Statistics

We used nonparametric statistics to test our data. Consequently, medians and percentiles are given for descriptive statistics and the Wilcoxon rank sum test is used for paired comparisons. Correlations are described with Spearmann correlation coefficients and corresponding P values. P values were considered significant when <0.05, nonsignificant (ns) when >0.05.

RESULTS

Results are presented on: (1) the epidermal thicknesses in the two test regions, (2) the relation between the epidermal thickness and the threshold dose to induce purpura, (3) the skin reflectance-evaluated redness, and (4) the occurrence of side effects.

Epidermal thicknesses in the two test regions. UVB-exposed buttock skin obtained thicker layers of stratum corneum, cellular epidermis, and total epidermis than the unirradiated buttock skin (Fig. 1).

Relation between epidermal thickness and threshold dose to induce purpura. There was no correlation between the epidermal thickness and the threshold dose to induce purpura 10 minutes and 1 day after laser exposure, neither for stratum corneum, the cellular part of epidermis, nor the total epidermis (r values ranged from −0.32–0.31, $P > 0.05$) (Fig. 2). Median values of the threshold doses were equal in the two test

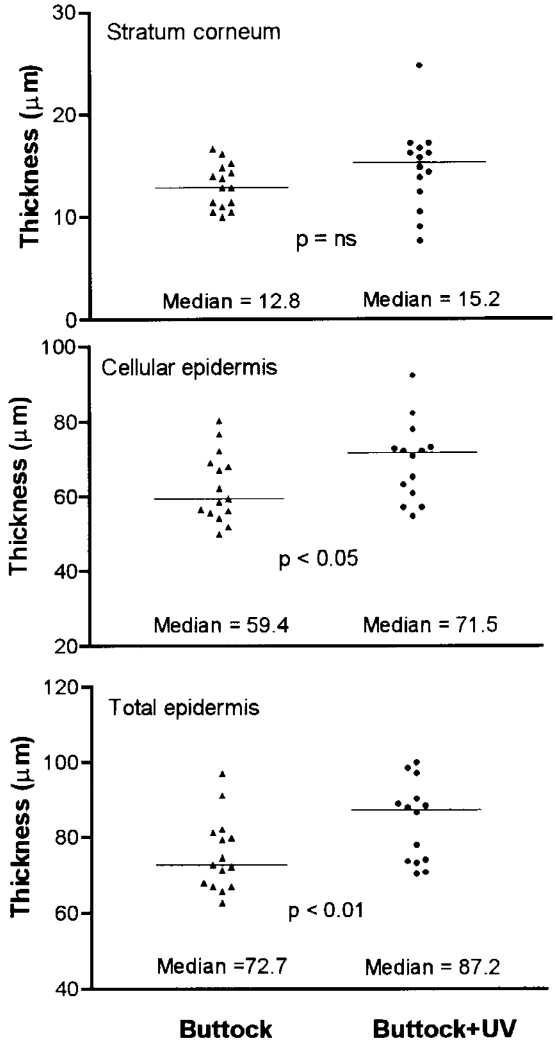

Fig. 1. The thicknesses of stratum corneum, cellular epidermis, and total epidermis are illustrated for the two test regions. The horizontal lines represent median values. The *P* values indicate the significance levels for unexposed buttock skin vs. UV-exposed buttock skin.

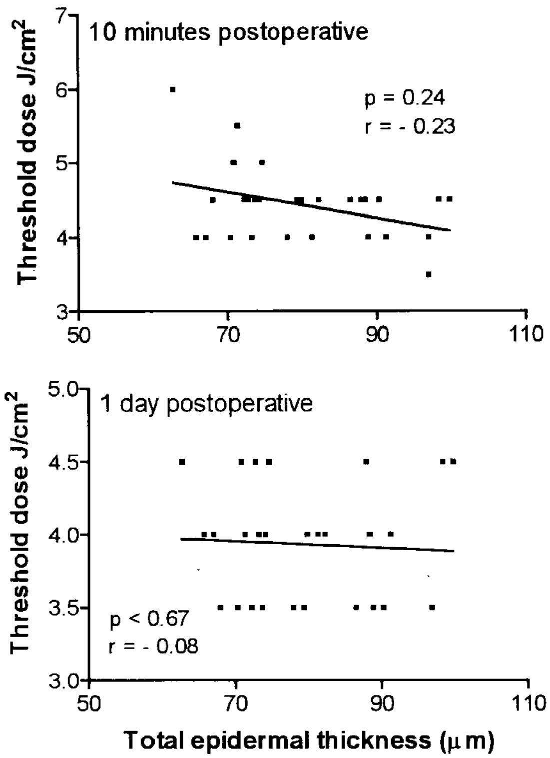

Fig. 2. The relation between total epidermal thickness and threshold dose to purpura 10 minutes and 1 day after laser exposure.

regions; 4.5 J/cm^2 10 minutes postoperatively (ns) and 4 J/cm^2 1 day postoperatively (ns).

Skin reflectance-evaluated redness. No correlation was seen between the total epidermal thickness and the skin reflectance evaluated redness at the definite fluence levels on 1, 6, and 22 days postoperatively. Overall, the skin redness showed a dose-response relation within the two test regions on 1 and 6 days after laser exposure (Fig. 3). The skin redness faded from day 1 to day 6, and furthermore to 2 weeks postoperatively, at which time the redness could be detected only at the highest fluences. Six weeks after laser exposure, there was no remaining laser-induced skin redness. It is the overall impression from Figure 3 that the UVB-exposed buttock region obtained higher redness degrees than the unexposed buttock region. Differences were significant at the 5 J/cm^2 treatment level (6, 22 days postoperatively) and at the 5.5 J/cm^2 treatment level (1, 6, 22 days postoperatively).

Occurrence of side effects. Clinical examination revealed no hypopigmentation, hyperpigmentation, epidermal alterations, atrophy, or hypertrophy in the three test regions. No changes in skin pigmentation were seen by skin reflectance.

DISCUSSION

In this study we have laser treated human volunteers in two test regions of varying epidermal thicknesses, using a model that is based on the fact that UVB-exposure induces epidermal hyperplasia and thus increases the epidermal thickness [9]. We failed to demonstrate that epidermal thickness is important to the clinically evaluated and skin reflectance-determined purpuric reaction after treatment with the PDL. Nevertheless, the skin reflectance evaluated skin

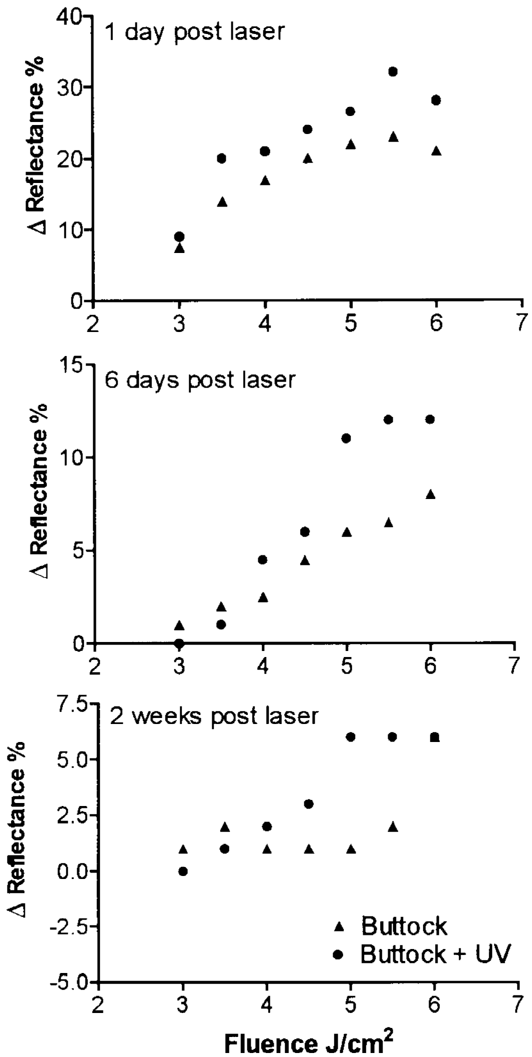

Fig. 3. Dose-response relations are illustrated for the two test regions on 1, and 6 days postoperatively. Each point represents a median value of the skin reflectance measurements Δ redness% - redness% laser treated skin - redness% normal skin. Note the decreasing range of values on the *y* axis. Negative values represent measurements of which the adjacent normal skin had a higher redness degree than the previously laser treated skin.

redness was more intense in the UVB-exposed buttock region (thick epidermis) than in the unexposed buttock region (thin epidermis). It seems unbelievable, however, that the purpuric reaction, i.e., the vessel specificity, should be reduced for skin with a thin epidermis as compared with skin having a thick epidermis. We, therefore, examined whether any confounding factors may have interfered with our results: At the time of laser exposure, no significant differences were seen between the two test regions regarding blood flow (UVB-exposed buttock (median 27, ranges 20–33)-unexposed buttock (25, 23–36) (ns)) and skin redness (UVB-exposed buttock (26%, ranges 23–29%)-unexposed buttock (27%, 24–29%) (ns)), whereas the skin pigmentation was slightly, but significantly higher in the UVB-exposed buttock region (median 8.5%, range 5–9%) than in the unexposed buttock region (5%, 3–8%) ($P < 0.0001$). However, the increased epidermal pigmentation does not give an explanation; on the contrary, it may have opposed the obtained results due to an increased threshold dose to induce purpura in pigmented skin [14]. Nevertheless, the increased pigmentation may also be responsible for a greater injury due to epidermal damage, resulting in an increased inflammation with an associated increased inflammatory erythema.

Overall, we do not have a precise explanation for the intense postoperative redness % in the UVB-exposed buttock region. However, UV-induced inflammatory mediators may have increased the reactivity in the test area as well as UV-induced alterations of the vascular bed may have interfered with our results. Therefore, before the laser treatment, we evaluated the histological appearance of the vessels in the two test regions of five volunteers by means of an indirect antigen-antistof technique for factor VIII-related antigen, staining for endothelium. Neither quantitative nor qualitative differences could be detected, and accordingly we consider histological variations in vessels of minor importance to our results.

The laser treatment of vascular lesions is optimally based on an unimpeded passage of laser light through the epidermal layers followed by selective targeting of hemoglobin, in this way allowing bleaching of the vascular lesion with a low occurrence of side effects. However, before targeting the intravascular hemoglobin, the laser light may be weakened, either by absorption in the overlaying melanin, by attenuation in the epidermal layers, or by scattering effects by dermal, collagen fibres. In the normal epidermis, melanin is mainly responsible for attenuation of the wavelengths in the visible spectrum, whereas the importance of variations in epidermal thickness decreases with increasing wavelength and is considered of minor importance in the visible part of the electromagnetic spectrum [15,16]. Nevertheless, from a clinical point of view, it is relevant to deal with the importance of epidermal thickness for several reasons. There is a considerable variation in thickness between and within individuals according to the anatomical region [17,18], the epi-

dermal thickness decreases with advancing age [19,20] and sun exposure is known to increase the thickness of the epidermal layers [9].

In our study the variation in total epidermal thickness ranged from 60–100 μm, and within this range we were not able to detect any influence on the clinically evaluated laser-induced purpura. These results are contrary to a previously published murine study in which we have demonstrated that increased epidermal thickness reduces the chronic side effects from treatment with a CVL at 578 nm, probably due to attenuation of the laser light [8]. However, the results from the PDL and the CVL are not directly comparable, since these represent a wide range of skin reactions obtained from respectively human and murine skin. The PDL represents a vessel-specific acute skin reaction due to the concept of selective photothermolysis and the CVL represents a chronic skin reaction from an unspecific coagulation necrosis. Moreover, the range of the total epidermal thickness was broader in the murine study (26–116 μm) than in the human study, indicating an explanation for the discrepancy between the acute and chronic results. It may be necessary to operate with a difference of >40 μm (60–100 μm) to show detectable differences on the importance of epidermal attenuation of laser light. However, the range of epidermal thicknesses from 60–100 μm approximates the clinical variations in epidermal thickness within laser-treated areas in humans. We, therefore, conclude that epidermal thickness is unimportant to the purpuric reaction after treatment with the PDL.

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